ROLE OF IMPRINT SMEAR IN BREAST LUMP

Dissertation submitted in partial fulfillment of regulation for the award of M.S. Degree in General Surgery

(Branch I)

The Tamilnadu
Dr. M.G.R. Medical University
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CERTIFICATE

Certified that this is the bonafide dissertation done by **Dr. R. RAJESWARI** and submitted in partial fulfillment of the requirements for the Degree of M.S., General Surgery, Branch I of The Tamilnadu Dr. M.G.R. Medical University, Chennai

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DECLARATION

I solemnly declare that the dissertation titled **"Role of imprint smear in breast lump"** was done by me from 2006 onwards under the guidance and supervision of **Professor Dr. A. RAMAMOORTHY M.S.**

This dissertation is submitted to The Tamilnadu Dr. MGR Medical University towards the partial fulfillment of the requirement for the award of MS Degree in General Surgery (Branch I).

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Period of Study	:	2006 - 2009
College	:	COIMBATORE MEDICAL COLLEGE
Dissertation Topic	:	ROLE OF IMPRINT SMEAR IN BREAST LUMP

The Ethics Committee, Coimbatore Medical College has decided to inform that your Dissertation is accepted / ~~Not accepted~~ and you are permitted / ~~Not Permitted~~ to proceed with the above Study.

Coimbatore - 14.

Date : 8.10.2007

Secretary
Ethics Committee

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Introduction

INTRODUCTION

The use of frozen section for intraoperative tissue diagnosis is a well accepted procedure. But it involves time and technology, which are lacking in many institutions.

Another method is the examination of the imprint of fresh specimens. This technique was favourably reported by DUDGEON and PATRICK (1927) and BAMFORTH and OSBORN (1958), but not until recently has achieved the recognition it deserves in the English literature. Despite its simplicity, speed and excellent cellular detail, we believe many centres are still not utilizing this technique to its full extent.

This study will help in assessing the value of intraoperative imprint cytology in comparison with preoperative FNAC and postoperative histopathology.

Aim of the study

AIM OF THE STUDY

1. To evaluate accuracy of intraoperative imprint cytology in breast lumps.

2. To compare intraoperative imprint cytology with that of preoperative FNAC and postoperative histopathological study.

3. To ascertain the value of imprint cytology especially its simplicity, accuracy, rapidity and cost effectiveness

Anatomy of breast

ANATOMY OF THE BREAST [17, 18]

DEVELOPMENT:

The epithelial lining of the ducts and acini of the breast is developed from ectoderm and the supporting tissue is derived from the mesenchyme. On each side of the ventral surface of young embryos, a thickened band of ectoderm develops (the milk ridge). It extends obliquely from the axilla to the inguinal region. In the human, the whole of this ridge disappears, excepting only a small portion in each pectoral region from which the breast arise. Accessory breast tissue will form along the course of the milk ridge if it dose not disappear outside the area where the breast normally develops.

Nipple is either flat or depressed at birth, but later projects beyond the surrounding skin.

The breast consists of glandular tissue (mammary gland proper) which secretes milk. It also has fibro-fatty tissue between its glandular lobes and lobules along with blood vessels, lymphatics and nerves covered by skin.

EXTENT (Fig.1)

Vertical: 2^{nd} to 6^{th} rib.

Horizontal: The side of the sternum to the midaxillary line.

About two thirds of the breast rests upon the pectoralis major, one third on the serratus anterior. At its lower medial quadrant the external oblique, separates it from the rectus abdominis. The breast lies in the subcutaneous tissue and is separated from the underlying muscles by the deep facia.

NIPPLE AND AREOLA:

Cylindrical (or) conical projection at the anterior mammary aspect at the level of 4^{th} intercostal space (nullipara). It is pink (or) dark in colour, traversed by 15-20 lactiferous ducts. It contains circular (erection) and longitudinal (retraction) muscle fibres.

Its base is encircled by a cutaneous discoidal area, the areola- rose pink in colour (nullipara). It becomes darker during pregnancy.

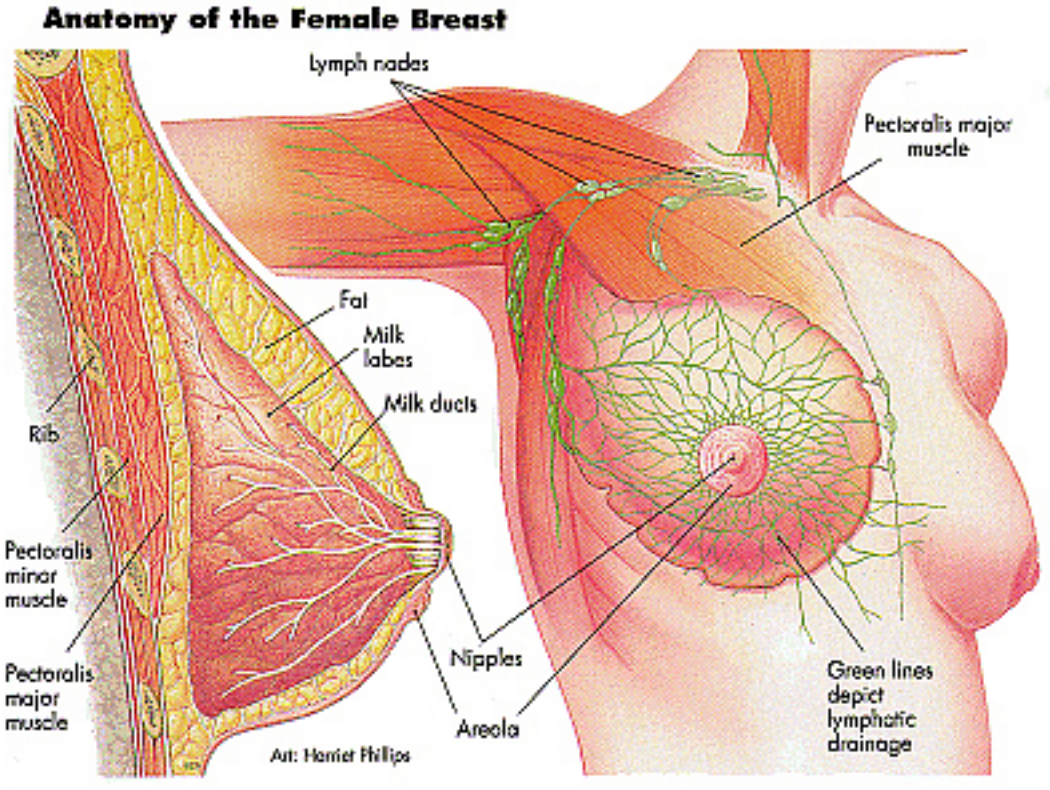

Anatomy of the Female Breast
Lymph nodes
Pectoralis major muscle
Fat
Milk lobes
Milk ducts
Rib
Pectoralis minor muscle
Pectoralis major muscle
Nipples
Areola
Green lines depict lymphatic drainage
Art: Harriet Phillips

FIG. 1

AXILLARY TAIL OF SPENCE:

This is a prolongation of outer part of the gland at the level of 3rd rib into axilla, where it is in direct contact with the main lymphnodes of the breast (anterior axillary lymphnodes).

This process of breast tissue gets into the axilla through an opening in the axillary fascia, known as the foramen of langer. The axillary tail is deep to deep fascia.

STRUCTURE OF BREAST (Fig.2)

It is composed of acini which make up lobules, aggregations of which form the lobes of the gland the lobes are arranged in a radiating fashion like the spokes of a wheel and converge on the nipple. Each lobe is drained by a duct, 10 -15 ducts open onto the nipple. The ducts are surrounded by connective tissue which is characteristically loose and vascular in the distal ductules.

Each portion of ducts involved in different disease.

1. Major duct - duct papilloma and duct ectasia.

2. Distal smaller ducts – fibroadenoma cyst formation and sclerosing adenosis.

3. Intra lobular portion of terminal ducts – carcinoma.

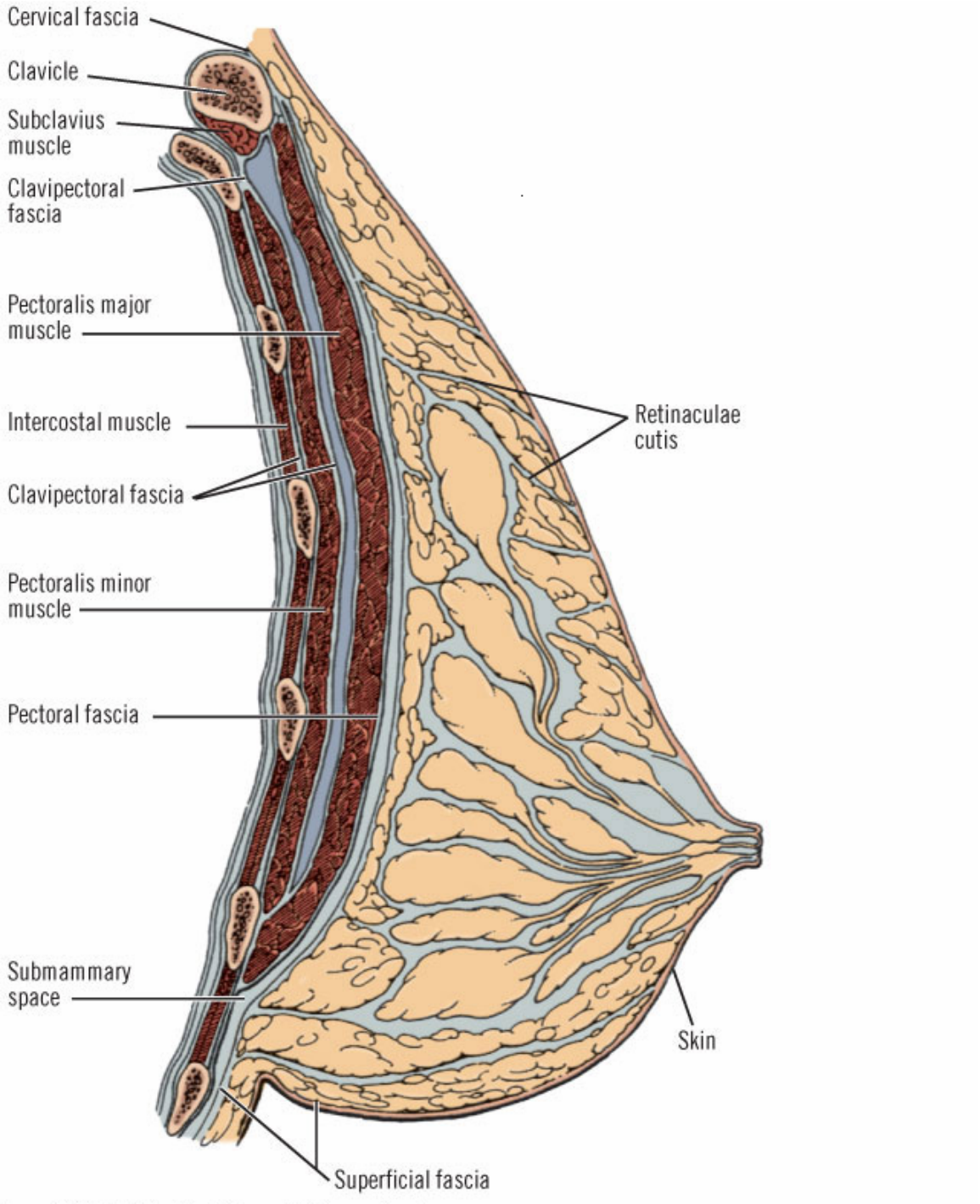


FIG. 2 ANATOMY OF BREAST

LIGAMENTS OF COOPER:

These are bands of connective tissue which anchors the skin on the pectoral fascia. Infiltration of this by malignant cells causes dimpling of the skin and fixity to the skin.

BLOOD SUPPLY (Fig.3)

Breast is supplied by

1) Lateral thoracic artery from 2^{nd} part of axillary artery.

2) $2^{nd,}$ 3^{rd} & 4^{th} perforators of internal mammary artery.

3) Lateral branches of 2^{nd}, 3^{rd} & 4^{th} intercostal arteries.

VENOUS DRAINAGE

It is to the axillary, internal mammary and intercostal veins.

NERVE SUPPLY (Fig.3)

Sympathetic nerves supply the secreting tissue via 2^{nd} to 6^{th} intercostal nerves. Skin is supplied by 4^{th} to 6^{th} intercostal nerves.

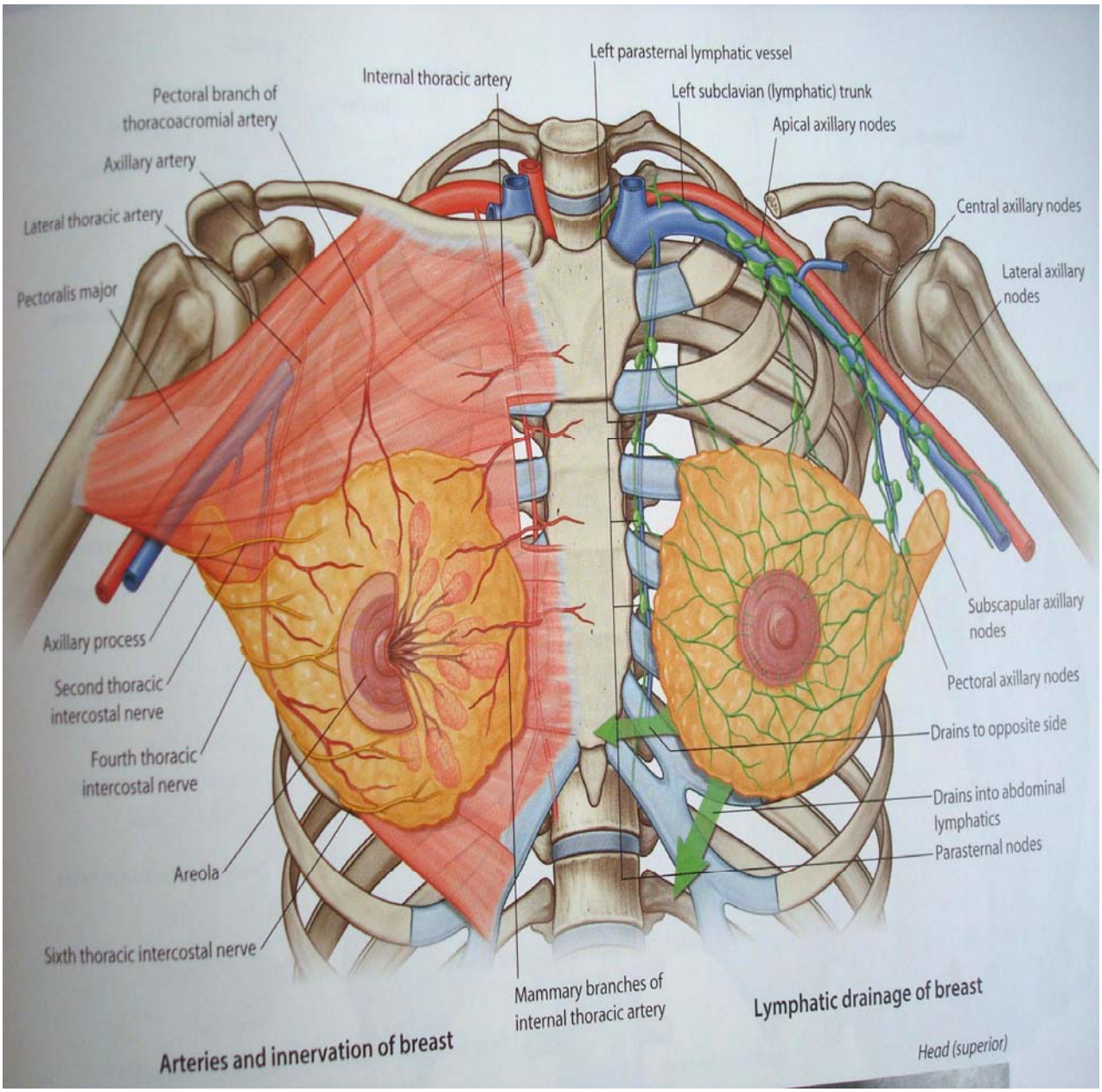


FIG.3 ANATOMY OF BREAST

LYMPHATIC DRAINAGE (Fig.4)

The breast is drained by two sets of lymphatics.

1. The lymphatics of the skin over the breast
2. The lymphatics of the parenchyma of the breast tissue.

Skin lymphatics of breast except areola and nipple drain into axillary nodes,

1. From the upper part to supraclavicular nodes.
2. From the medial part to internal mammary nodes.

The subareolar lymph plexus of sappey is a collection of large lymph vessels under the areola.

Most of the lymphatic go to anterior group, few pass to the posterior group, from there to central and apical group.

From the deeper surface, vessels pierce through pectoral muscle to the axillary and internal mammary nodes.

From the medial and inferior part of breast lymph vessels drain into internal mammary nodes.

At the level of first intercostal space, fine lymphatics connect right and left internal mammary chains behind the manubrium sterni.

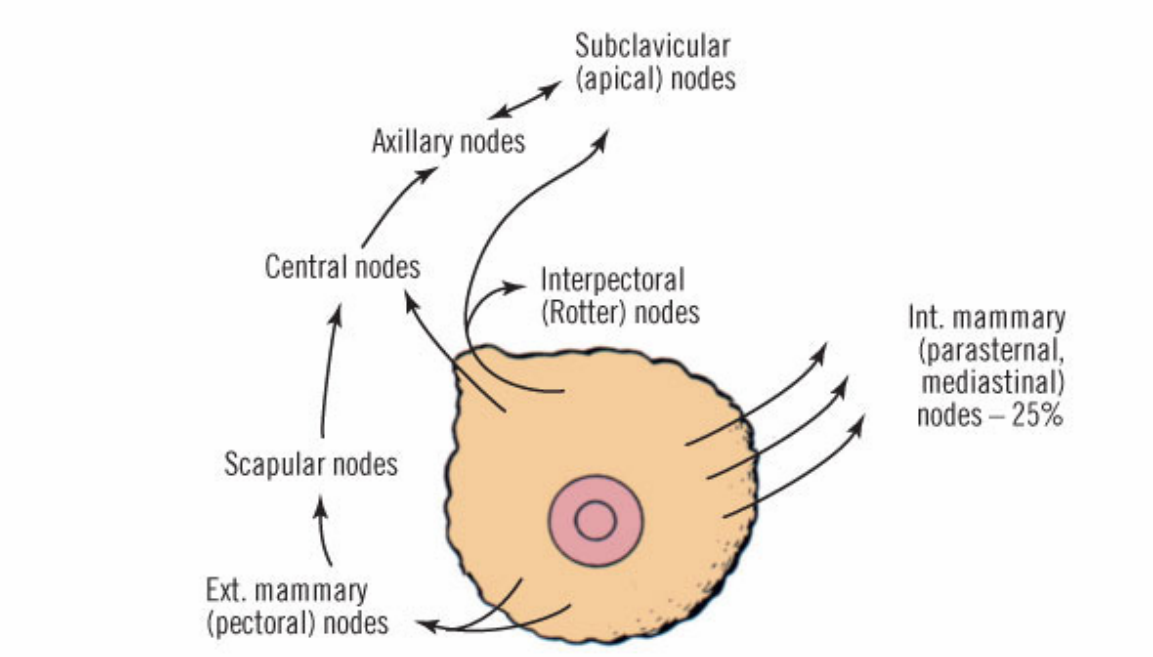


FIG. 4 LYMPHATIC DRAINAGE

85% of the lymphatic drainage of the breast is to the axillary nodes. There were five groups in axillary nodes.

1. Anterior group:

They situated along the lateral thoracic vein under the anterior axillary fold. They lie mainly on the 3rd rib. The axillary tail of Spence is in actual contact with those nodes and, therefore, cancer involving this process may be misdiagnosed as an enlarged node with an apparently healthy breast and the anterior axillary nodes may be involved, by direct continuity of tissue.

2. Posterior group:

These lie along the posterior axillary fold in relation to the subscapular vessels.

3. Lateral group:

It lies along the upper part of the humerous in relation to the axillary vein.

4. Central group:

It is situated in the fat of the upper part of the axilla. The intercostobrachial nerve passes outwards amongst these nodes. Enlargement

of these nodes, such as occurs in cancer may, by pressure on the nerve, cause pain in the distribution of the nerve along the inner border of the arm.

5. Apical group:

These are also called the infraclavicular nodes. They are very important and constant in position being bounded below by the 1st intercostal space, behind by the axillary vein, in front by the costocoracoid membrane. These lie very deeply, but can be palpated by pushing the fingers of one hand into the axillary apex from below, and the fingers of the other hand behind the clavicle from above.

Clinical and pathological aspects

CLINICAL / PATHOLOGICAL ASPECTS [21, 22, 23]

A lump in the breast may have different etiopathogenesis and clinical presentation.

MASTITIS WITH ABSCESS (BREAST ABSCESS):

This will have signs of infection and inflammation cytology may show pus cells and other leucocytes. Culture of the pus will be positive for the causative organism.

Chronic intramammary abscess following an inadequate drainage or injudicious antibiotic treatment, when encapsulated it is difficult to distinguish it from carcinoma. Antibioma usually culture negative.

DUCT ECTASIA / PERIDUCTAL MASTITIS:

Dilatation of the breast ducts associated with periductal inflammation. There may be discharge from the nipple which is greenish in colour. Single or multiple ducts may be involved.

HAEMATOMA:

In this condition the diagnosis is by either aspiration or incision. History of trauma causing the hematoma will be involved.

FAT NECROSIS:

It follows a trauma. It requires biopsy to differentiate it from carcinoma, as it clinically mimics malignancy due to its consistency.

ABERRATIONS OF NORMAL DEVELOPMENT & INVOLUTION (ANDI):

This is due to changes in breast throughout a women's reproductive life and cyclical changes. Clinically there may be a lump or pain.

Pathologically the breast is white or yellow and rubbery in consistency. Microscopically there may be

1) Cyst formation.
2) Fibrosis. Fat and elastic tissue replaced by fibrous tissue.
3) Hyperplasia of epithelium lining the ducts with atypia.
4) Papillomatosis. There is papillomatous overgrowth within the ducts.

CYST:

It occurs in the last decade of reproductive life due to non integrated involution of stroma and epithelium. It may be bilateral and multiple, diagnosed by aspiration and ultrasonography.

GALACTOCELE:

It always follows lactation. It is a solitary cyst containing milk. Lymphatic cyst and hydatid cyst may occur as cystic lump.

DUCT PAPILLOMA:

Usually single. Age group 35-40yrs. It usually has dark blood stained discharge from nipple.

Histologically the papillary connective tissue axis is covered by cuboidal or cylindrical epithelial cells.

FIBROADENOMA:

Age group 18 – 25yrs. It usually encapsulated freely mobile in the breast tissue (breast mouse).

The stroma is usually delicate, cellular, and often myxoid, resembling intra lobular stroma, enclosing glandular and cystic spaces lined

by epithelium. The epithelium may be surrounded by stroma or compressed and distorted by it. In older women, the stroma typically becomes densely hyalinized and the epithelium atrophic.

PHYLLODES TUMOUR (Fig.5)

This is a large tumour often having bulbous protrusions due to the presence of nodules of proliferating stroma covered by epithelium. They are distinguished from fibroadenoma on the basis of cellularity, mitotic rate, nuclear pleomorphism, stromal overgrowth, and infiltrative borders. Low grade lesions resemble fibroadenoma but with increased cellularity and mitotic figures. High grade lesions may be difficult to distinguish from sarcomas.

CARCINOMA

NON INVASIVE (INSITU) CARCINOMA

A) INTRADUCTAL CARCINOMA (DCIS):

With the advent of Mammography, it now constitutes 22-80% of carcinomas. It is defined as malignant population of cells that lack the capacity to invade the basement membrane and incapable of distant metastasis.

FIG.5 PHYLLODES TUMOUR

There are five subtypes,

1) comedocarcinoma (more malignant).
2) Solid.
3) Cribriform.
4) Papillary.
5) Micropapillary.

B) LOBULAR CARCINOMA INSITU (LCIS):

There is proliferation of loosely cohesive cells in one or more terminal ducts / acini. It is frequently multifocal and bilateral. It is a marker for invasive carcinoma.

INVASIVE CARCINOMA (Fig.6, 7)

A) INVASIVE DUCTAL CARCINOMA:

This is the common type. Incidence is 65-80% of all mammary cancers. There may be nipple retraction, dimpling of skin or fixity to the chest wall muscles.

Histologically malignant duct lining cells disposed in cords, solid cell nests, tubular glands or anastamosing masses.

FIG.6 EARLY CANCER LT BREAST

FIG.7 LOCALLY ADVANCED CANCER RT BREAST

B) MEDULLARY CARCINOMA:

Incidence 1-5% donot have striking desmoplasia. Histologically solid syncytium like sheets of large cells with lymphocytic infiltration is present.

C) COLLOID OR MUCINOUS CARCINOMA:

Occurs in old women, slow growing, histologically large lakes of amorphous mucin with scattered neoplastic cells are present.

D) PAGET'S DISEASE (Fig.8)

It involves the nipple. There is invariably underlying ductal carcinoma insitu. Histological hallmark is involvement of epidermis by malignant cells.

E) INVASIVE LOBULAR CARCINOMA:

Peculiar feature is bilateral and multicentric. Histologically, it consists of strands of infiltrating tumour cells, loosely disposed throughout the fibrous matrix.

FIG.8 PAGET'S DISEASE LT BREAST

F) SARCOMAS:

Usually solid tumours, there may be cystic degeneration. Examples of sarcomas are fibrosarcoma, liposarcoma & stromal sarcoma. Histologically spindle cells are seen.

G) LYMPHOMAS:

Primary lymphomas are rare. There is predominance of diffuse histiocytic lymphomas.

H) INFLAMMATORY CARCINOMA :

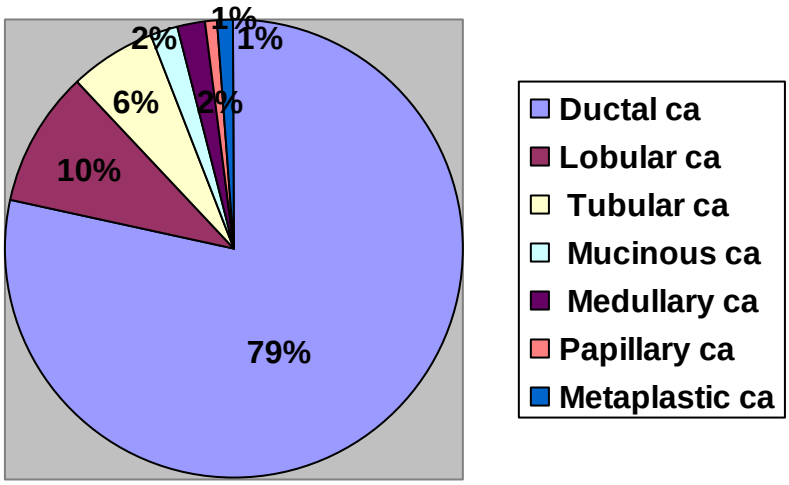
This carcinoma is usually mistaken for breast abscess. Clinically erythema, peau d' orange will be there. The subdermal lymphatics and vascular channels are permeated by tumour cells. Polymorphs and lymphocytes are absent near the tumour. It has fast metastatic potential.

Incidence of histological types of breast cancer [24]

INSITU CARCINOMA	-15-30%
Ductal carcinoma insitu	-80%
Lobular carcinoma insitu	-20%

INVASIVE CARCINOMA	-70-85%
Invasive ductal carcinoma	-79%
Invasive lobular carcinoma	-10%
Tubular carcinoma	-6%
Colloid/mucinous ca	- 2%
Medullary carcinoma	- 2%
Papillary carcinoma	-1%
Metaplastic carcinoma	-<1%

The above distribution is charted as follows



Normal breast cytology

CYTOLOGY OF NORMAL BREAST [20]

The breast in mature women consist of 15 – 20 lobes surrounded by fibrous and adipose tissue with each lobe containing multiple secretary acini and lobules embedded in a dense intralobular stroma. The lobes drain separately through lactiferous ducts to the nipple. Lobules and ducts are lined by columnar epithelial cells which appear as a double (or) pseudostratified layer in the acini and stratified squamous towards the nipple.

DUCT CELLS:

The duct cells are arranged in tight groups and sheets. The nuclei are oval (9-12 nm in the long axis) with coarse chromatin and sparse blue cytoplasm. Few individual duct cells are seen within a group; the cells show only moderate variation in size.

APOCRINE CELLS:

These are duct cells which have undergone apocrine metaplastic change. Apocrine glands which are similar to sebaceous glands, are normally found in the axilla, the breast is a modified apocrine organ and

when is stimulated by hormones, some of the ductal cells show a dramatic increase in the amount of cytoplasm (the so called pink epithelium). This epithelium is unusual in the normal breast but is common in fibrocystic hyperplasia.

Apocrine cells are large cells (30-50nm) with an oval (or) round nucleus similar in size to duct cells with a looser chromatin.

There will be increase in cytoplasm, cytoplasmic granules and the frequent binucleate cells.

STRIPPED NUCLECI:

As their name implies these cells do not have any cytoplasm. They appear as oval nuclei (8 - 10 mcg in the long axis) with smooth homogenous chromatin single or in pairs. They are either myoepithelial cell nuclei or nuclei from the intralobular connective tissue. Presence of stripped nuclei strongly suggests that the lesion is benign.

FOAM CELLS:

These are large cells with abundant foamy cytoplasm. The nuclei are degenerated usually pyknotic and often absent. Multinucleation and considerable variation in cell size is common. They are derived either from duct lining cells or histiocytes. If foam cells are present the lesion is likely to be benign.

FAT CELLS:

Clumps of adipose tissue cells occur with large well defined cell borders. Clear unstained cytoplasm and small dark eccentric nuclei. They are of no significance.

HISTIOCYTES AND GIANT CELLS:

Show a gross variation in cell size and can mimic other cells. They have a variably shaped nucleus which may be round, oval or indented with paranuclear chromatin. There is a variable amount of pale gray/ blue cytoplasm often having a frosted glass appearance. The cell borders are poorly defined but the cells appear single rather than in groups. The giant cell is a fusion of histiocytic cells forming syncytium. Histiocytes and giant cells are seen in variety of conditions in the breast both benign and malignant, but are commonly found in fat necrosis. If giant cells are present it does not exclude malignancy but the lesion is probably benign.

BLOOD CELLS:

Leucocytes and red cells are often seen. Large numbers of neutrophils are usually indicative of a breast abscess. Lymphocytes may be present in large number around carcinoma.

Abnormal breast cytology

BREAST CYTOLOGY IN ABNORMAL CONDITIONS [19, 20, 24, 25]

FIBROADENOSIS (Fig.9)

This lesion typically produces a twin cell population of duct cells and stripped nuclei. The duct cells occur in moderately sized groups and sheets showing tight adhesion one to another with the stripped nuclei scattered in between.

Among the duct cells epithelial cells of oxyphil or apocrine types are found in addition to the usual bimodal cell population of ductular epithelium and single bare nuclei. The nuclei are round, nuclear size may vary considerably and nucleoli are prominent.

FIBROADENOMA (Fig. 10)

This lesion can be suggested as a diagnosis when the groups of duct cells are very large and numerous with many stripped nuclei scattered throughout. The age of the patient is of paramount important as a specimen of this cellularity in an older woman would be suspicious of carcinoma, although in this situation stripped nuclei would be absent.

Criteria for diagnosis-

1. A high cell yield.

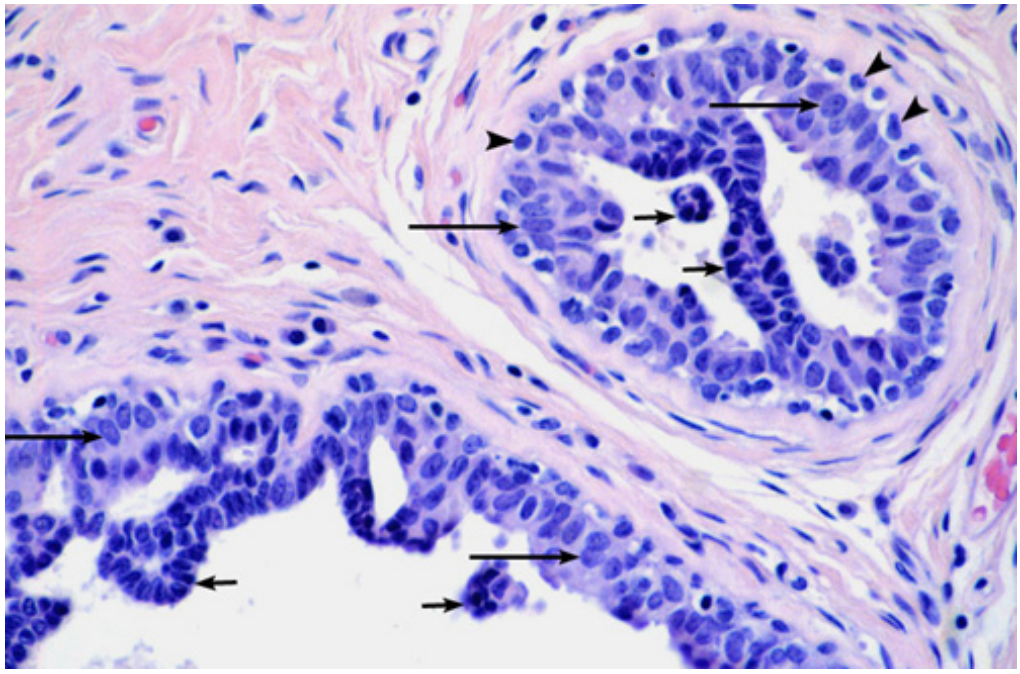


FIG.9 FIBRO CYSTIC DISEASE

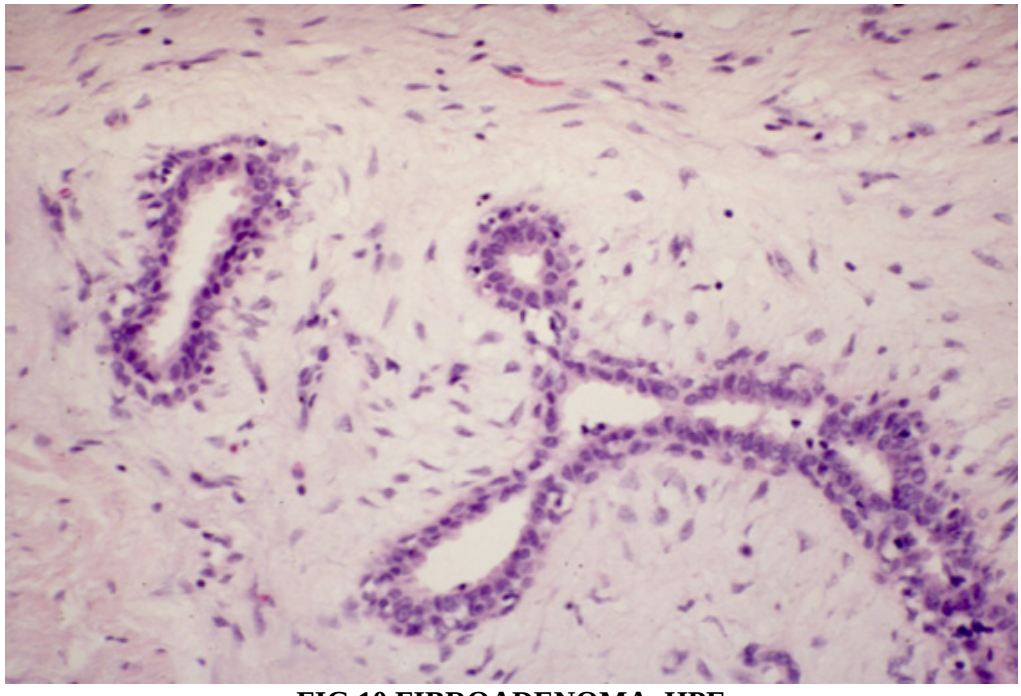


FIG.10 FIBROADENOMA HPE

2. Large, branching, monolayered sheets of uniform epithelial cells.

3. Numerous single, bare nuclei of benign type

4. Fragments of fibromyxoid stroma.

Smears show a bimodal pattern with increased cellularity. The nuclei of these cells are larger than the normal cells. Epithelial hyperplasia especially if atypical often accompanies malignancy and if seen in a FNAC requires mammographic examination and followup.

PREGNANCY AND LACTATION (Fig.11)

When examining the aspirated material in pregnancy and lactation, especially in the third trimester some of the criteria for malignancy are seen. The specimen has a high cellularity, there is loss of adhesion of the cells which are larger than normal and pleomorphic. However the uniformly round nuclei and particularly the many lipid droplets seen in the background material are typical of lactation and benign. The nuclear chromatin is finely granular and evenly distributed.

FNAC in pregnancy is difficult to interpret and unless full patient details are supplied by the clinician, a false positive result is a very real danger.

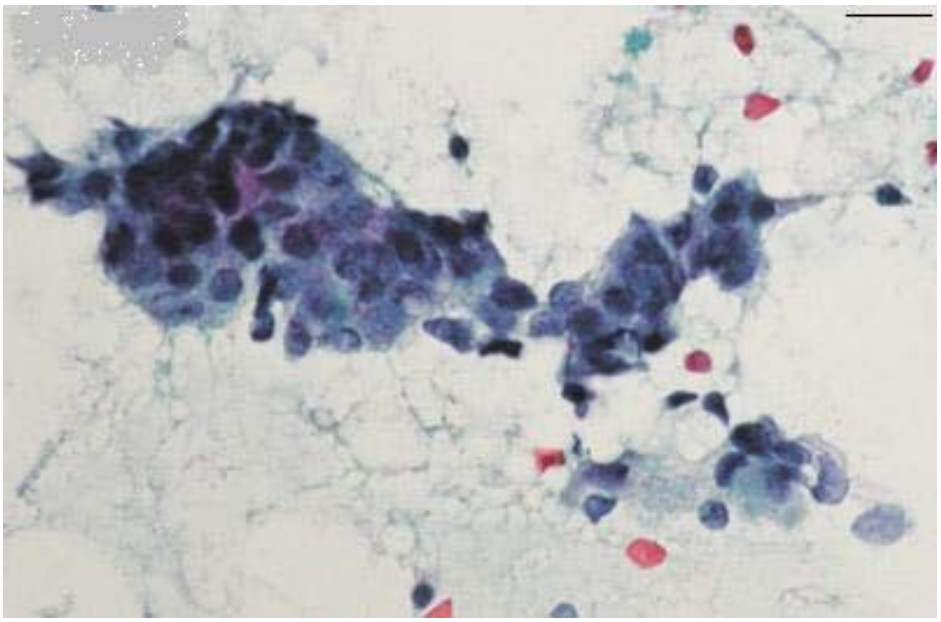


FIG.11 LACTATING BREAST

FAT NECROSIS:

A mixed picture of debris, red blood cells, degenerate pus cells, foam cells, histiocytes (most containing lipid droplets) and degenerate adipose tissue.

These cells tent to occur singly rather than in the usual pattern of adherent groups. Giant cells may be present. Ducts are not usually seen. Fat necrosis may be acute (mostly pus cells) or healing (histiocytes and giant cells). Its distinctive appearance once seen is easily recognized again.

1. A background of granular debris, fat and fragments of adipose tissue.
2. Foamy macrophages, multinucleated giant cells and adipocytes with bubbly cytoplasm.
3. Chronic inflammatory cells.
4. Absence of epithelial cells.

INFLAMMATION:

Acute mastitis produces sheets of degenerative pus cells and other leucocytes, usually with histiocytes scattered throughout. Bacteria may also be present. Duct cell shows nuclear enlargement and degeneration due to inflammation if they present. There is loss of

adhesion which may suggest malignancy. But acute inflammatory cells commonly rarely associated with carcinoma of the breast.

MASTITIS:

The common findings are

1. A benign bimodal pattern.
2. Inflammatory cells, chronic and (or) acute.
3. Regenerative epithelial atypia.
4. Epitheloid histiocytes, multinucleated giant cells and many plasma cells (granulomatous mastitis).

Cytological findings in nonspecific granulomatous mastitis and in tuberculous mastitis have been described.

SUBAREOLAR ABSCESS:

There is mature (or) anucleated squmous cells in greater numbers may be due to contamination from skin.

PHYLLODES TUMOUR (Fig.12, 13)

It has a similar cytological appearance to fibroadenoma but the stromal elements predominate over the epithelial elements. Single bare nuclei are numerous. In some cases cohesive fragments of highly cellular stroma of spindle cells may be found.

CARCINOMA OF BREAST:

A comparison of benign and malignant features in breast cytology;

BENIGN	**MALIGNANT**
1. Good cell adhesion to one another. 2. Cellularity low. 3. Frequent stripped nuclei. 4. Normal cell size. 5. Uniformity of cells. 6. Coarse but regular nuclear chromatin.	1. Loss of cell adhesion. 2. cellularity high. 3. Lack of stripped nuclei. 4. Increased cell size. 5. Pleomorphism. 6. Variable nuclear chromatin often with prominent nuclei occasionally a lymphocyte response.

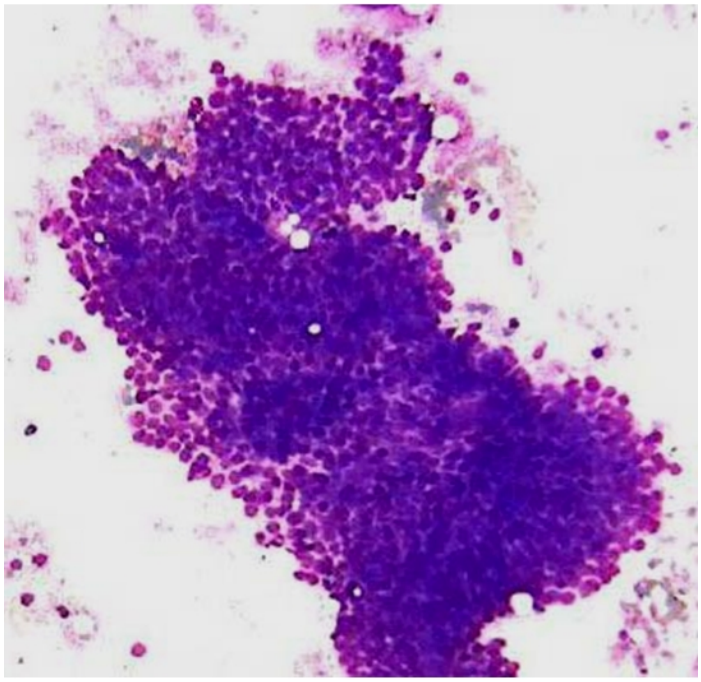


FIG.12 PHYLLODES CYTOLOGY

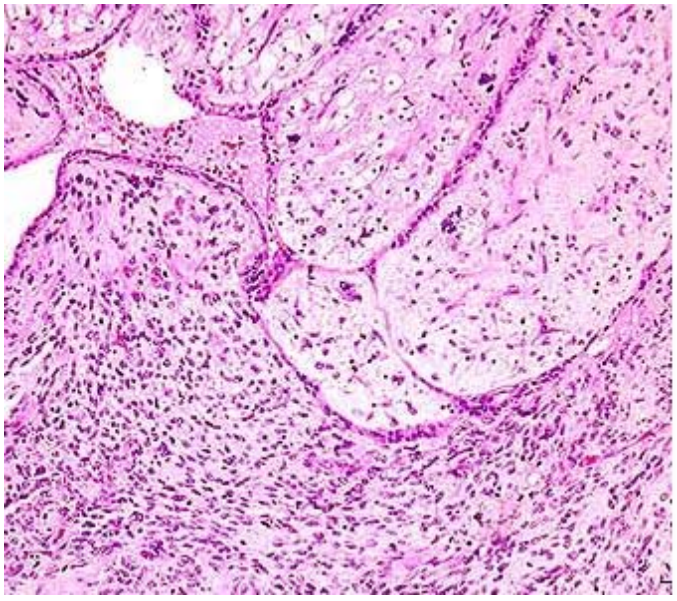


FIG.13 PHYLLODES HPE

DUCTAL CARCINOMA INSITU (DCIS):

COMEDO CARCINOMA:

Usual findings are

1. Variable cell yield.
2. Neoplastic cells in irregular aggregates and singly.
3. Large pleomorphic cells showing obvious malignant features.
4. Necrotic debris lymphocytes and vacuolated macrophages. The cells are large and pleomorphic vacuolated macrophages contain hemosiderin pigments represents the comedo plugs. Calcification may be seen.

PAPILLARY TYPE:

It is highly cellular smear papillary aggregates with central fibrovascular core. Columnar cells in row, palisades and single. Bare nuclei of benign type are absent. Variable nuclear enlargement, pleomorphism and atypia are seen. Necrotic debris is also present.

CRIBRIFORM:

Epithelial cells relatively cohesive forming large monolayered sheets seen. Mild epithelial atypia and necrotic debris is seen. Macrophages often with hemosiderin pigment are present.

LOBULAR CARCINOMA INSITU (LCIS):

It is difficult to define cytological criteria for this diagnosis in FNA smear. Intra cytoplasmic neolumina is the most useful clue to the cytological diagnosis.

INFILTRATING DUCTAL CARCINOMA (Fig.14)

Criteria for diagnosis,

1. High cell yield.
2. A single population of atypical epithelial cells.
3. Irregular angulated clusters of atypical cells.
4. Reduced cohesiveness of epithelial cells.
5. Nuclear enlargement and irregularity.
6. Single cell with intact cytoplasm.
7. Absence of single bare nuclei.
8. Necrosis, important if present.

A scirrhous cancer may yield very few cells (or) no cells at all. Nuclei are irregular in shape and irregular in contour, which is diagnostic of malignancy. However the cell population can be quite monomorphous and nuclear abnormalities may be suitable.

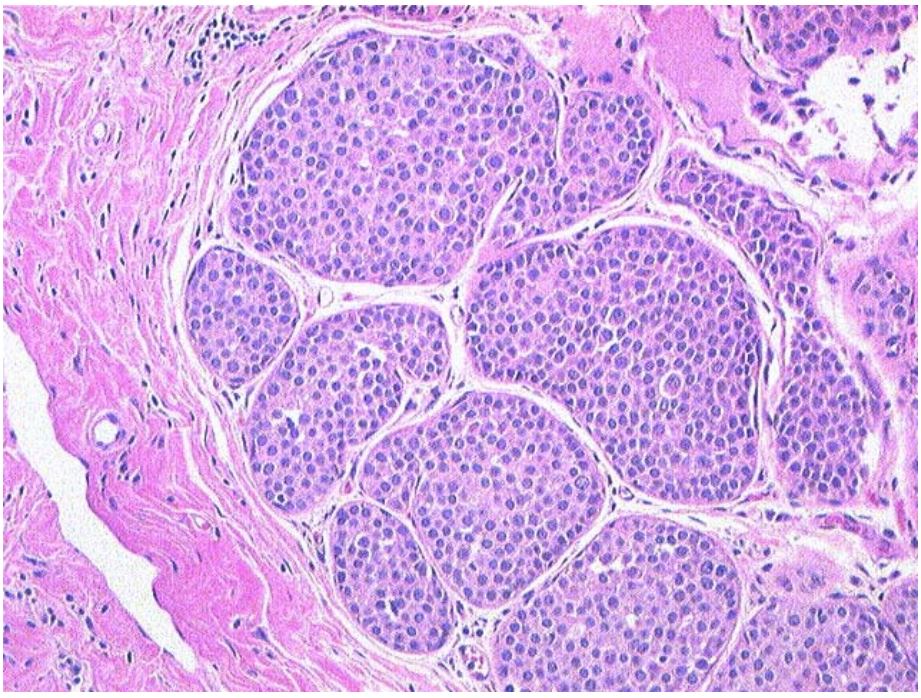


FIG.14 INVASIVE DUCTAL CARCINOMA HPE

In poorly differentiated carcinoma dissociation of cells may be total and smear may resemble large cell lymphoma. Single carcinoma cell usually have well defined cytoplasm but infiltrating lobular carcinoma is an exception.

TUBULAR CRACINOMA:

It shows moderate cellular smears. Cells are predominantly in cohesive clusters. Epithelial clusters with an angular shape and tubular pattern is present. Single bare nuclei are often present.

MUCINOUS CARCINOMA (Fig. 15, 16)

It has a soft empty feel to the needle while doing FNAC. It often bleeds easily. The cytology is quite similar to comedocarcinoma. The smear is highly cellular. Irregular cell aggregates and single cells with lymphocytic infiltration are seen. Nuclei is large and pleomorphic.

INFILTRATING LOBULAR CARCINOMA (Fig. 17)

It has an abundant desmoplastic stroma. The cells are seen in small groups and single file. The cells have often lost their cytoplasm and often show nuclear moulding. The other findings are poor cell yield. Cytoplasm

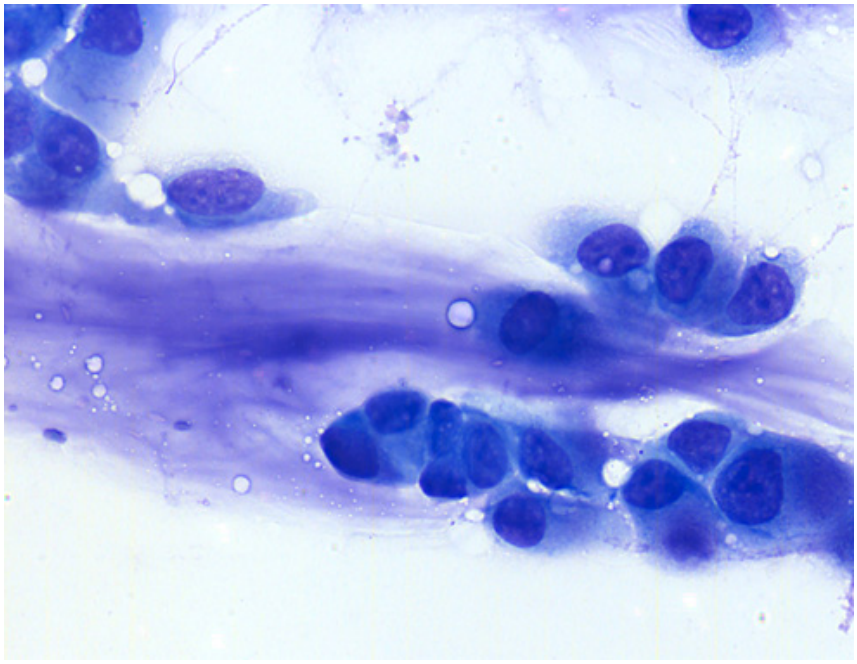


FIG.15 COLLOID CARCINOMA CYTOLOGY

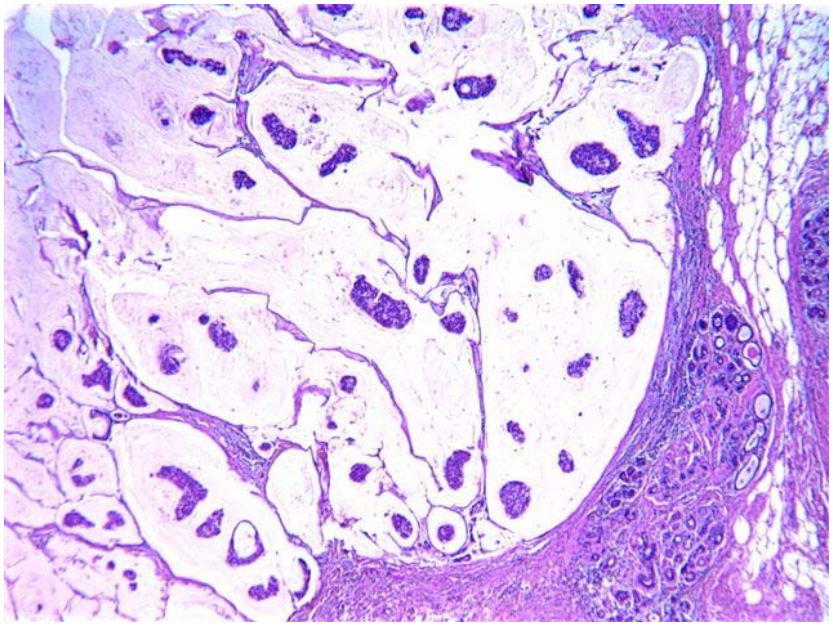


FIG.16 COLLOID CARCINOMA HPE

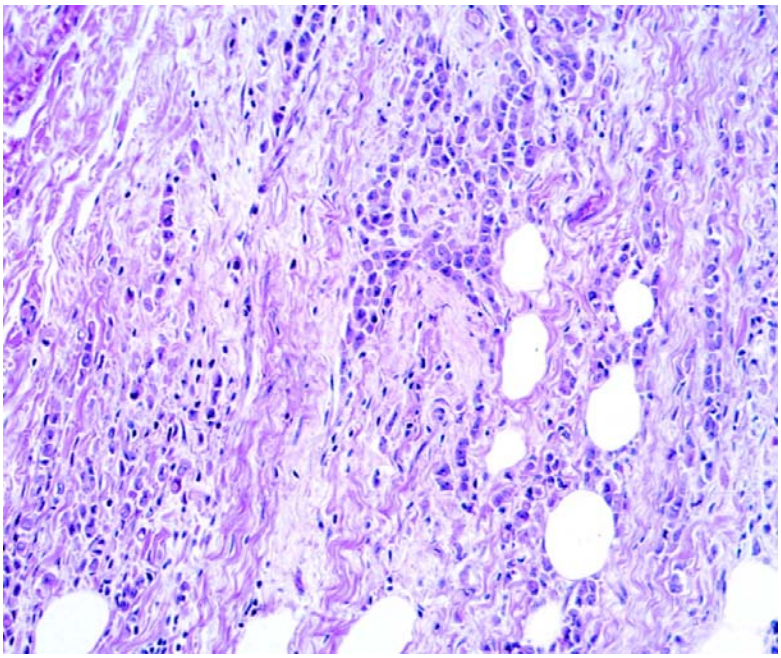


FIG.17 INVASIVE LOBULAR CARCINOMA HPE

will be scanty and indistinct. There are cells with small dark nuclei and irregular in shape.

PAGET'S DISEASE OF NIPPLE:

Background shows keratin, squamous cells and inflammatory cells, large malignant cells. There will be abundant pale cytoplasm.

INFLAMMATORY CARCINOMA:

There is thickening and erythema of the skin due to extensive intralymphatic spread of tumour causing lymph stasis and edema. The cytological pattern is similar to that of the common carcinoma. Inflammatory cells are not seen.

SARCOMA:

Smear from a tumour contain fragments of highly cellular stromal tissue of the spindle cells which may show nuclear atypia and pleomorphism. There is variable number of sheets of epithelial cells. However definite diagnosis is by histological examination. In angiosarcoma plenty of blood cells and tumour cells may be few in number. Most cells are in syncytial cluster but some are single.

Techniques

TECHNIQUES

FINE NEEDLE ASPIRATION CYTOLOGY[20]

It is most often used for diagnosis of palpable mass lesions like breast mass, enlarged lymph nodes, enlarged thyroid, superficial soft tissue masses, salivary gland, palpable abdominal lesions and testicles. Other sites accessible to FNAC are prostate, pelvic organs, bone and joint spaces, lungs, retroperitoneal tumours and orbit.

PROCEDURE:

Materials: 1. Needles:

a) 21 G disposable needles of 38mm length are suitable for routine transcutaneous FNAC.

b) 25 (or) 24G disposable needles of 25mm length for lymph nodes and children.

c) 80 – 160mm length needles are required for lungs and abdominal viscera.

d) 22 – 20G Chiba spinal puncture needles of upto 200mm length are used for transrectal, transvaginal FNAC of the prostate and ovary.

e) 18G needles for bony lesions.

f) 21G needles for superficial cystic lesions.

2) Syringe: 10 -20 ml syringe with Franzen handle.

3) Glass slides and fixatives.

METHODS OF ASPIRATION

Patient kept in lying position and target area well exposed. Target area thoroughly palpated and cleaned with an alcohol pad. Mass was fixed by the palpating hand and the needle was inserted into the target area (Fig18). On reaching the lesion the plunger was retracted and at least 10ml of suction applied while moving the needle back and forth within the lesion. The direction of the needle may be changed to access different areas of the lesion. Aspiration was terminated when aspirated material (or) blood becomes visible at the base of the needle. On completion of aspiration, suction was released and pressure within the syringe allowed to equalize before withdrawing the needle.

Following with drawl of the needle from the lesion, pressure is applied to the site of puncture.

Aspirated material is now recovered by detaching the needle from the syringe and filling the syringe with air. The syringe and needle are then reconnected and the aspirate expressed onto one end of a glass slide.

After preparing smear, it was air dried and stained with eosin and haematoxylin and examined under microscope (Fig.19, 20).

FIG.18 FNAC TECHNIQE

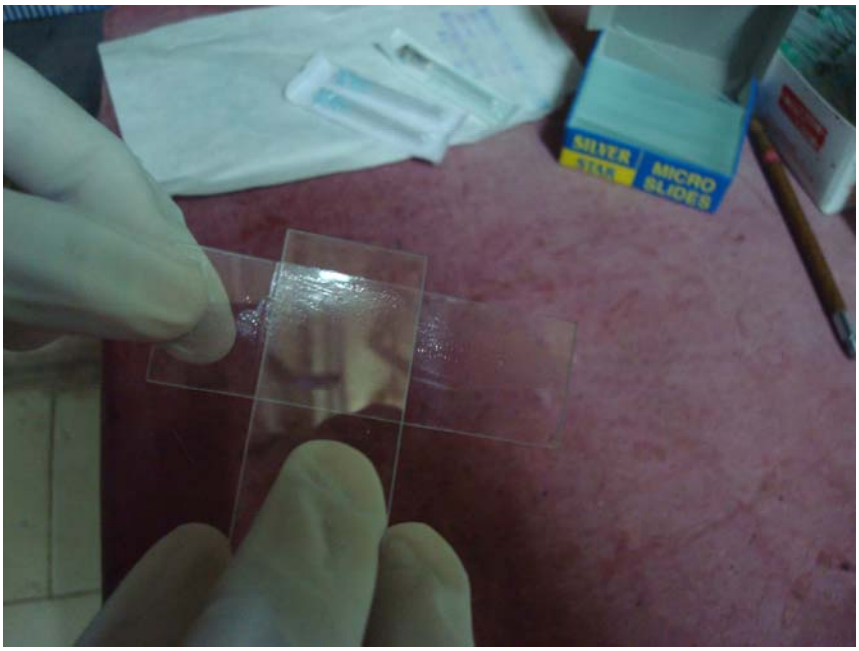


FIG.19 SMEARING

FIG.20 LABLING AND STAINING

ADVANTAGES:

There is no hospitalization, no anaesthesia, quick, safe and is painless. Multiple attempts are possible without inconvenience. Results can be obtained rapidly within 2 – 24 hrs. It is a low cost procedure and special studies can be done.

Special stains: Wet fixed smears are used for a variety of special stains such as Alcian blue, mucicarmine and PAS (for amyloid) and bacterial and fungal stains (for infectious agents). Cell block, immunocyto chemical studies, immuno analysis, morphometry, and flow cytometry can be done. It is determination of ploidy status and S phase fractions of tumour cells using flow cytometry enhances the diagnostic and prognostic information available on routine cytology.

Ultra structural studies: Aspirates obtained by FNAC are also suitable for electron microscopy. Molecular biologic techniques – detection of oncogenes like ERBB 2 in breast cancer and BCC 2 in lymphomas has been reported in aspiration samples.

USG, CT or MRI guided FNAC also can be done.

COMPLICATIONS

It includes haematoma, Infection and dissemination of tumour

LIMITATIONS:

Main limitation of FNAC is that only small population of cells sampled by the procedure. The reliability of the test depends upon the adequacy of the sample. Inadequate sample which is not representative of the true lesion results in false negative diagnosis. If the FNAC report is negative despite a strong clinical suspicion of malignancy the patient should be investigated further. FNAC may be repeated (or) a surgical biopsy performed to obtain a tissue diagnosis in such instances.

Causes of unsatisfactory yield are (Fig.21)

1. Needle has missed the target tangentially.
2. Needle in central cystic/nectrotic/haemorrhagic area devoid of diagnostic cells.
3. Needle in a dominant benign mass missing a small adjacent malignant lesion.
4. Fibrotic/desmoplastic target tissue giving a scant cell yield.

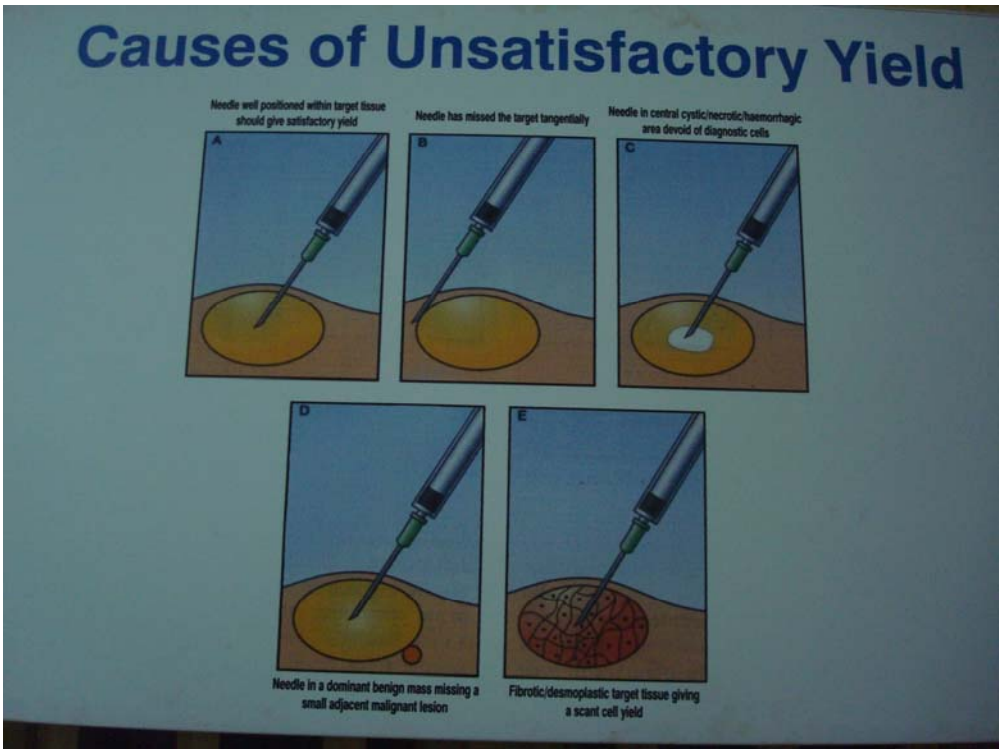

Causes of Unsatisfactory Yield
Needle well positioned within target tissue should give satisfactory yield
A
Needle has missed the target tangentially
B
Needle in central cystic/necrotic/haemorrhagic area devoid of diagnostic cells
C
D
Needle in a dominant benign mass missing a small adjacent malignant lesion
E
Fibrotic/desmoplastic target tissue giving a scant cell yield

FIG.21

IMPRINT TECHNIQUE [20, 25]

After preparing the patient for general anesthesia either excision of the lump (or) mastectomy done according to the clinical diagnosis and FNAC report (Fig.22).

The specimen which was freshly cut opened was kept in one hand with flat surface upward (Fig.23).

With the other hand lightly touch an alcohol clean glass slide repeatedly in serial adjacent areas with the cut surface of the tissue (Fig.24). Compression of the tissue was avoided as too much pressure may distort the cells morphology. Mere contact with the slide is sufficient.

If the surface touched is excessively bloody (or) wet, the slide was discarded and repeated with another slide until the touch preparation are barely opaque. An average of four slides prepared.

Each slide was prepared and air dried. It takes only 30 to 60 seconds for the slide to dry. If it takes more it means that the touches are too wet and that the resulting imprint will be unsatisfactory.

After drying the smear was fixed with isopropylalcohol (Fig.25) and stained with eosin and haematoxylin staining technique. After staining, the slide was examined by the cytopathologist under the microscope.

FIG.22 BREAST LUMPECTOMY

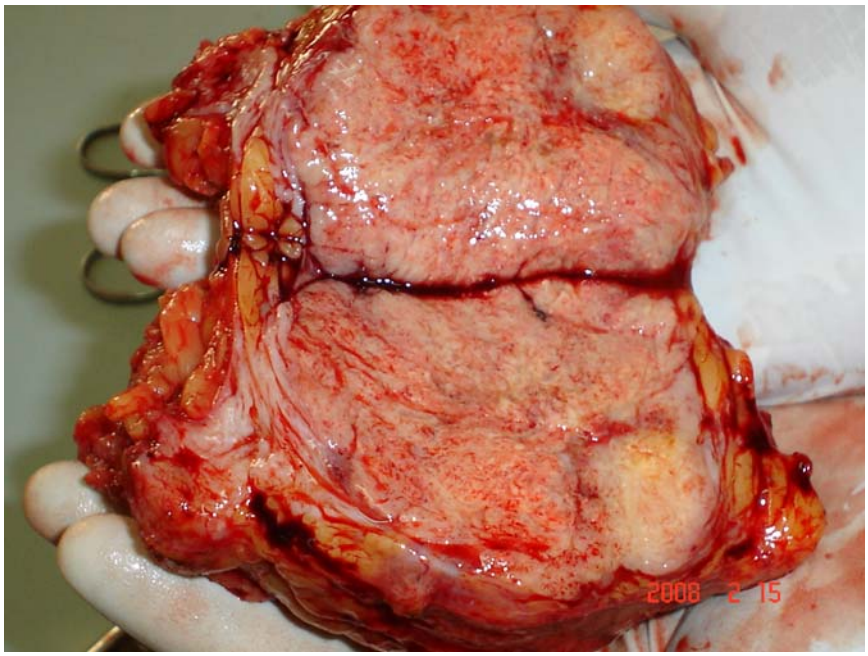


FIG.23 BREAST LUMP – CUT SECTION

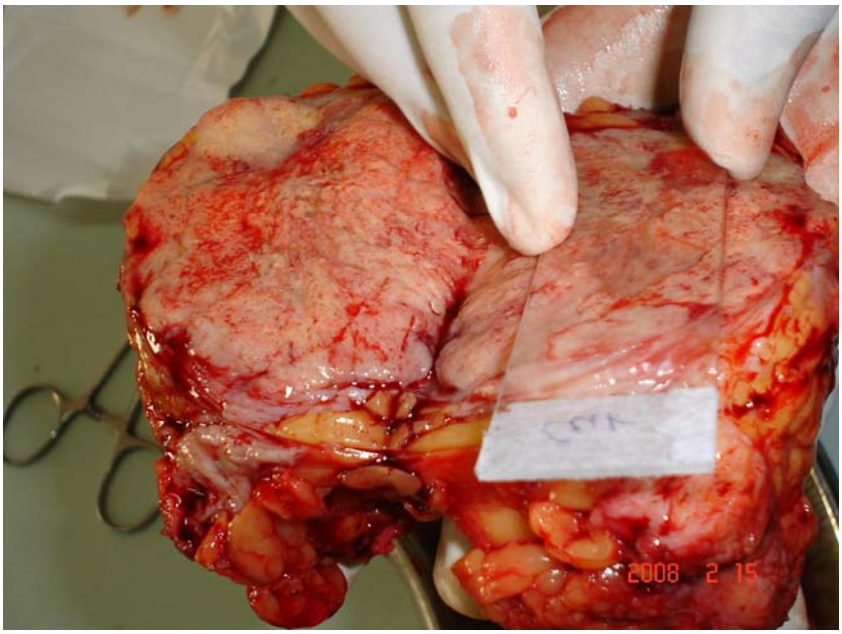


FIG.24 IMPRINT TECHNIQUE

FIG. 25 COUPLIN JAR

Wet fixed smears are used for a variety of special stains such as Alcian blue, mucicarmine and PAS (for amyloid) and bacterial and fungal stains (for infectious agents). Cell block, immunocyto chemical studies, immuno analysis, morphometry, and flow cytometry can be done like FNAC. Ultra structural studies are also possible. Molecular biologic techniques – detection of oncogenes like ERBB 2 in breast cancer and BCC 2 in lymphomas has been reported in imprinted cells.

HISTOTECHINIQUE[20]

Tissue specimens received in pathology lab are accessioned by giving them a number that will identity each specimen for each pt.

Gross Examination:

Gross examination consists of describing the specimen & placing all (or) parts of it into small plastic cassette which holds the tissue while it is being processed to a paraffin block. Initially the cassettes are placed into a fixative.

Fixation:

Fixation is used to preserve the tissues permanently. Fixation should be carried out as soon as possible after removal of the tissues to prevent autolysis. There is no perfect fixative though formaldehyde comes closer.

Tissue processing (Fig.26)

Once the tissue has been fixed, it must be processed into a form

in which it can be made into thin microscopic section. The usual way this is done with paraffin tissues embedded in paraffin which is similar in density to tissues, can be sectioned at any where from 3 to 10 microns usually 6-8 routinely. The technique of getting fixed tissue into paraffin is

called tissue processing. The main steps in this processing are dehydration and clearing.

Sectioning:

Once the tissues have been embedded, they should be cut into sections that can be placed on a slide. This is done with a microtome (Fig27).Once sections are cut they are floated on a warm water bath (Fig.28) that helps to remove wrinkles. Then they are picked up on a glass microscope slide. After picking up the tissue dewaxing done (Fig.29).

Staining:

The staining process makes use of variety of dyes, that have been chosen for their ability to stain is that of hematoxylin and eosin. Other stains are referred as special stains.

Cover slipping:

The stained section on the slide should be covered with a thin piece of glass to protect the tissue from being scratched to provide better optical quality for viewing under the microscope and to preserve the tissue section for years to come.

FIG.26 PARAFFIN BLOCKS

FIG.27 MICROTOME

FIG.28 WARM WATER BATH

FIG.29 INCUBATOR FOR DEWAXING

Materials and methods

MATERIALS AND METHODS

This study was a prospective study including patients, who underwent elective breast surgeries for breast lump in the DEPARTMENT OF GENERAL SURGERY, COIMBATORE MEDICAL COLLEGE HOSPITAL, COIMBATORE from 2006-2008. 50 female patients with breast lump except breast abscess and cystic lesions were included in this study.

METHOD OF COLLECTION OF DATA

All the female patients undergoing elective surgeries for breast lump were evaluated as per the proforma with a thorough history, clinical examination and all necessary investigations. FNAC was done routinely to get the preoperative cytological diagnosis. Surgery was planned according to the cytological study. Based on FNAC diagnosis all benign breast lumps underwent excision, malignant breast lump underwent modified radical mastectomy, total mastectomy (or) toilet mastectomy according to stage.

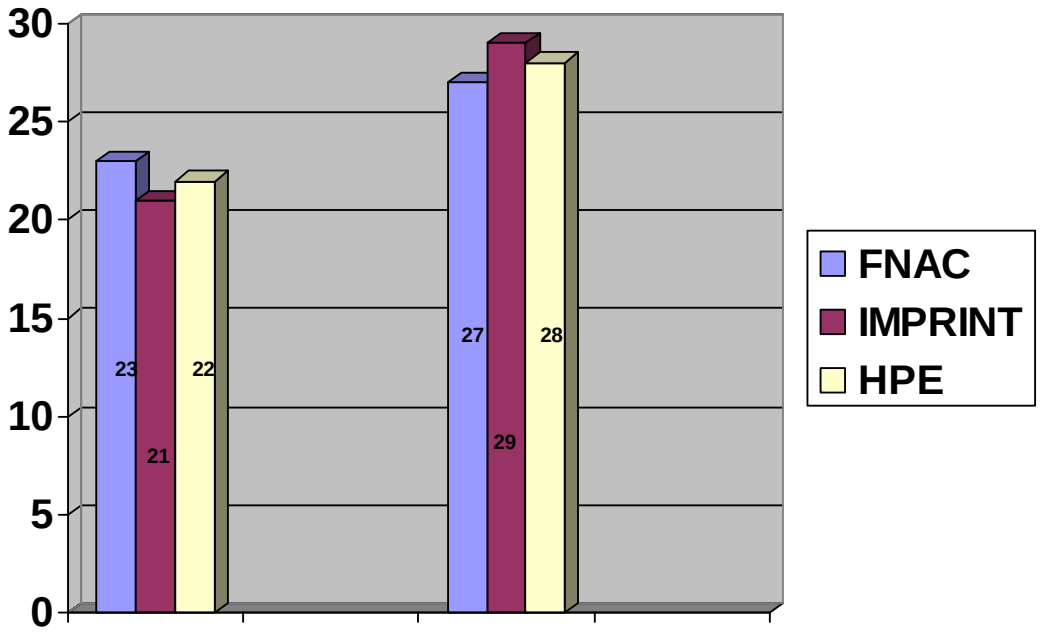
Immediately after excision cut section was done at the level of the lump and the cut surface was touched over the previously cleaned glass slide and fixed in isopropyl alcohol after air drying. After rapid

haematoxylin and eosin staining the smear was interpreted by the cytopathologist.

Results of the study

RESULTS

	BENIGN	MALIGNANT
FNAC	21+(1- Inconclusive)	27+(1 False negative)
Imprint	21	28+(1 False positive)
HPE	22	28



BENIGN MALIGNANT

RESULTS OF THIS STUDY

	Imprint	FNAC	HPE
Sensitivity	100%	96.4%	100%
Specificity	95.4%	100%	100%
Positive predictive value	96.5%	100%	100%
Negative predictive value	100%	95.7%	100%
False positive	4.5%	0%	0%
False negative	0%	3.6%	0%

Discussion

DISCUSSION

Common breast masses include, Fibroadenoma, Fibrocystic lesions, Cyst &malignancy.

Breast masses management is primarily based on three important criteria like, Physical examination of breast, Breast imaging &Breast FNAC or biopsy.

The ultimate and gold standard diagnosis is by submitting the breast mass for histopathological examination after excision. Preoperative diagnosis was by breast imaging and FNAC. But they remain inconclusive if the representative sample is not obtained preoperatively, it becomes essential to decide whether the mass is benign or malignant and also to decide whether the cut margins of a suspected malignant mass are involved by malignant cells which would indirectly influence the amount of excision or surgery.

Per operative decision was made by either frozen section biopsy or imprint cytology. Imprint cytology is ahead of frozen section in decision making and is being used in some major hospitals and teaching centers.

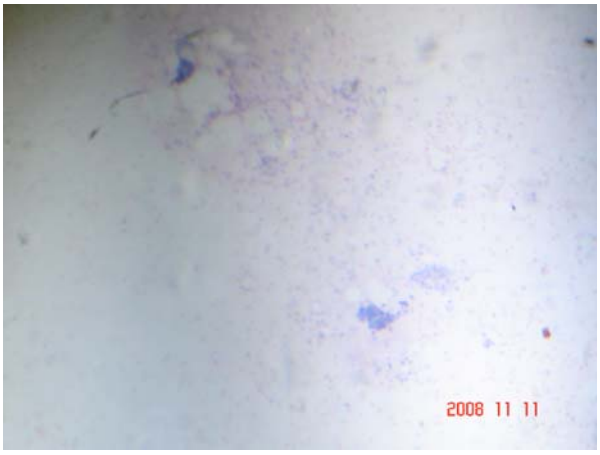
This study was comparative study to ascertain the usefulness of imprint cytology in diagnosis of breast swelling and whether the imprint cytological findings concur with the FNAC done preoperatively and

regular histopatological examination done postoperatively. 50 female patients with breast mass were taken up for this study from all units in the Department of surgery. Fig.30, 31 shows microscopic view of FNAC, Imprint, HPE of a fibroadenoma and ductal carcinoma respectively.

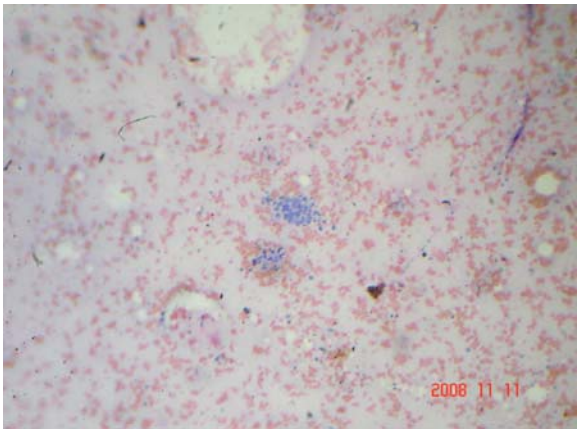
All patients underwent FNAC and there were 22 patients with benign mass and 27 patients with malignant mass according to FNAC. One of the patients FNAC was inconclusive. There was one false negative in the FNAC taken.

In the imprint cytology done all these patients, 21 patients had benign lesion whereas 29 patients showed malignancy. One of the patients who was described to have malignant lesion was later found to have a benign phyllodes tumour (as per regular HPE study). There by constituting the false positive study. One case mentioned as malignant phyllodes was metaplastic ca as per HPE. As the metaplastic ca includes adenocarcinoma with a chondroid stroma, squmous cell ca and carcinoma with prominent spindle cell component it might be difficult to distinguish from sarcomas[20].

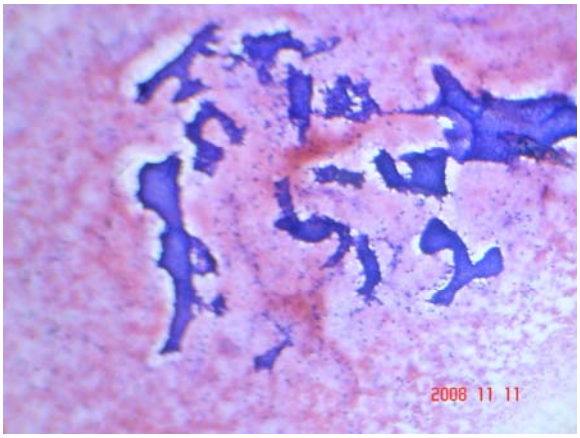
Lobular ca not made out in case of mixed type (ductal & lobular). Valdes concluded in his study that intraoperative imprint had limited value for intraoperative assessment of margins for invasive lobular ca[28].



FIBROADENOMA FNAC

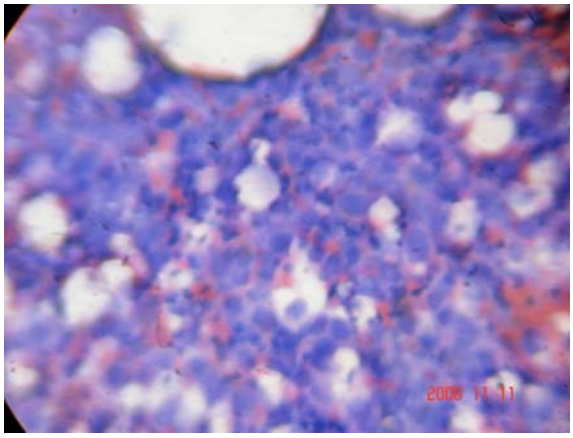


FIBROADENOMA IMPRINT

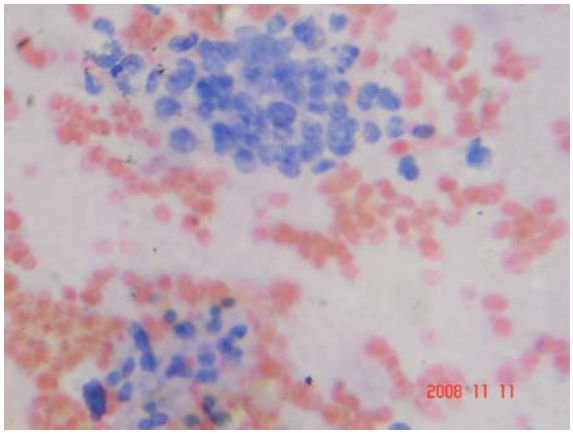


FIBROADENOMA HPE

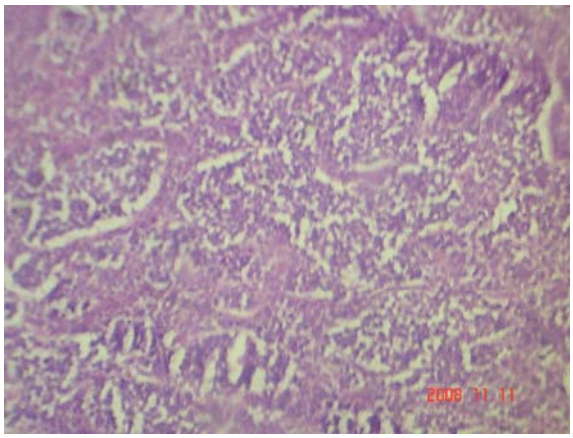
FIG.30



DUCTAL CARCINOMA FNAC



DUCTAL CARCINOMA IMPRINT



INVASIVE DUCTAL CARCINOMA HPE
FIG.31

One case which was inconclusive in FNAC was also missed in regular cuts of HPE. After further cuts the case was diagnosed as intraductal papilloma.

One case false negative in FNAC was diagnosed by tru-cut biopsy preoperatively, was also diagnosed in intraoperative imprint smear.

Statistical index of diagnostic accuracy defined as the ability of a test to identity correctly all those who have the disease that is “True Positive”.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + false negative}} \times 100 = \frac{28}{28+0} \times 100 = 100\%$$

Defined as the ability of a test to identify correctly those who do not have the disease, that is “True Negative”.

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative + False positive}} \times 100 = \frac{21}{22} \times 100 = 95.4\%$$

The performance of a screening test is measured by its predictive value which reflects the diagnostic power of the test. The predictive accuracy depends upon sensitivity, specificity and disease prevalence.

Predictive value of positive test:

$$\frac{\text{True positive}}{\text{True positive and False positive}} \times 100 = \frac{28}{29} \times 100 = 96.5\%$$

Predictive value of negative test:

$$\frac{\text{True negative}}{\text{False negative + True negative}} \times 100 = \frac{21}{22} \times 100 = 100\%$$

The term false negative means that patients who actually have the disease are told that they do not have the disease. It amounts to giving them a “false reassurance”.

Percentage of false negative

$$\frac{\text{False negative}}{\text{True positive + False negative}} \times 100 = \frac{0}{28 + 0} \times 100 = 0\%$$

The term false positive means that patients do not have the disease are told that they have disease. In this case normal healthy people may be subjected to further diagnostic test at some inconvenience, discomfort, anxiety and expense until their freedom from disease is established.

Percentage of false positive

$$\frac{\text{False positive}}{\text{False positive + True negative}} \text{X100} = \frac{1}{1+21} \text{X100=4.5\%}$$

Our study results were correlating with other study results.

Abhijit D, Hiregoudar reported that accuracy rate for benign lesion was 100% and that for malignant lesion was 97.5% with a false negative rate of 2.5%. Then sensitivity, specificity, positive predictive value and negative predictive value were 95.24%, 100%, 100% and 95% respectively. He concluded that intraoperative imprint smears like frozen section helps in on table diagnosis, wherein the fine needle aspiration cytology is in conclusive (or) suspicious[1].

K.C.Suen reported the overall accuracy rate for this imprint cytology was 93.8% with a false positive rate of 0.24% with a false negative rate of 6%[2].

KU reported a sensitivity of 100% specificity of 97.1% and diagnostic accuracy of 97.7% for imprint cytology[6].

Cox reported a sensitivity of 100% and specificity of 97%[13].

Khanna reported a sensitivity of imprint in breast lump in breast lump 98.4% a specificity of 100%[3].

Veneti reported a sensitivity of 97.1% a specificity of 99.4% and accuracy of 98.3% for imprint cytology[7]

Klimberg reported a sensitivity of 100% and specificity of 100%[14].

Kim reported the efficacy of intraoperative imprint cytology to be superior to frozen section analysis[5]

Scopa reported as accuracy rate of 94.3%. He suggested that intra operative pathologic evaluation of margin status may be useful to reduce the need for re excision[4].

Dutta reported that the combination of FNAC and imprint smear gave a diagnostic accuracy of 96%[10].

England assessed the adequacy of wide local excision of breast cancer using specimen scrape cytology and tumour bed biopsy[8].

Shirham in reported a strong favour for imprint cytology during intraoperative consultation[11].

Creager concluded that the sensitivity and specificity of imprint cytology are similar to that of frozen section evaluation[12].

Pinotti concluded that intraoperative pathological monitoring of surgical margins is a safe and accurate method to prevent additional surgery for insufficient margins and to reduce the recurrence rate[9].

Shiver SA, concluded that sensitivity and specificity of imprint are similar to that of frozen section evaluation. Imprint cytology is therefore a

viable alternative to frozen section when intraoperative evaluation is required[15].

Winberg E , concluded that intraoperative imprint cytology provides an accurate evaluation of lumpectomy margins for patients undergoing breast conservation treatment[16].

Conclusion

CONCLUSION

In the present study the Imprint cytology more or less correlates with FNAC and definitely a useful peroperative evaluating procedure. Imprint cytology comes handy in places where frozen section facilities are not available.

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Proforma

PROFORMA

NAME: **AGE/SEX:** **I.P NO:**

ADDRESS:

COMPLAINTS:

MENSTRUAL HISTORY:

MARITAL HISTORY:

OBSTETRIC HISTROY:

FAMILY HISTORY:

CLINICAL BREAST EXAMINATION:

CLINICAL DIAGNOSIS:

FNAC:

TREATMENT:

IMPRINT SMEAR:

HPE:

COMPARISION:

MASTER CHART							
~~S.NO~~	**NAME**	**IP.NO**	**AGE**	**CLINICAL DIAGNOSIS**	**FNAC**	**IMPRINT SMEAR**	**HPE**
~~1.~~	Rajalakshmi	71763	29	fibroadenoma	fibroadenoma	fibroadenoma	fibroadenoma
2.	Geethakumari	72325	27	fibroadenoma	fibroadenoma	fibroadenoma	fibroadenoma
3.	Sangeetha	608	18	fibroadenoma	Fibrocystic disease	Fibrocystic disease	fibroadenoma
4.	Lilly	612	55	Duct papilloma	inconclusive	Duct papilloma	ductal papilloma
5.	Palaniyammal	1028	62	ca breast	Ductal ca	Ductal ca	Ductal ca
6.	Sulochana	76509	45	Ca breast	Ductal ca	Ductal ca	Ductal ca
7.	Krishnammal	1154	76	Ca breast	Ductal ca	Ductal ca	Ductal ca
8.	Mary	1965	44	Ca breast	Ductal ca	Ductal ca	Ductal ca
9.	Jeya lakshmi	2411	42	Ca breast	Ductal ca	Ductal ca	Ductal ca
10.	Rathinam	3194	55	Ca breast	Benign pattern	Ductal ca	Ductal ca
11.	Kalavathi	3562	35	fibroadenoma	fibroadenoma	fibroadenoma	fibroadenoma
12.	Ruthmary	4466	32	fibroadenoma	fibrodenoma	fibroadenoma	Gian fibroadenoma
13.	Valliyammal	1304	51	Ca breast	Ductal ca	Ductal ca	Ductal ca
14.	Meharaj	4018	39	Ca breast	Breast ca	Ductal ca	Ductal ca
15.	Mary	5673	59	ca breast	Ductal ca	Ductal ca	Ductal ca
16.	Thulasi	6375	50	ca breast	Ductal ca	Ductal ca	Ductal ca
17.	Malish	7423	33	fibroadenoma	fibroadenoma	fibroadenoma	fibroadenoma
18.	Thennarai	5251	40	ca breast	Ductal ca	Ductal ca	Ductal ca
19.	Rayathal	6766	67	ca breast	Ductal ca	Ductal ca	Pappilary ca
20.	Susheela	7593	36	fibroadenoma	Benign pattern	fibroadenoma	fibroadenoma
21.	Lillygracy	9514	43	ca breast	Fibrocystic disease	Fibrocystic disease	Fibrocystic disease
22.	Mani	6153	43	ca breast	Fibrocystic disease	Fibrocystic disease	Fibrocystic disease
23.	Sundarambal	10036	60	ca breast	Ductal ca	Ductal ca	Ductal ca
24.	Selvi	9193	46	Phyllodes tumour	Phyllodes tumour	malignancy	Phyllodes tumour
25.	Shailaja	10446	50	ca breast	Ductal ca	Ductal ca	Ductal ca

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~~26.~~	Shanthi	10696	45	fibroadenoma	fibroadenoma	fibroadenoma	fibroadenoma
27.	Pappammal	10119	65	Ca breast	Ductal ca	Ductal ca	Ductal ca
28.	Kaliyammal	10546	37	Phyllodes tumour	Phyllodes tumour	Phyllodes tumour	Phyllodes tumour
29.	Mary	11987	23	fibroadenoma	fibroadenoma	fibroadenoma	fibroadenoma
30.	Suganthi	12649	24	Fibroadenoma	Fibrocystic disease	Fibroadenoma	Fibroadenoma
31.	Noorjahan	10286	45	Ca breast	Fibrocystic disease	Fibrocystic disease	Fibroadenoma
32.	Sheela	11566	40	Ca breast	Ductal ca	Ductal ca	Ductal ca
33.	Lakshmi	13378	50	Ca breast	Ductal ca	Ductal ca	Ductal ca
34.	Shanthi	11550	33	Ca breast	Ductal ca	Ductal ca	Ductal ca
35.	Palaniyammal	8736	49	Ca breast	Ductal ca	Ductal ca	Ductal ca
36.	Palayee	14568	42	Ca breast	Ductal ca	Ductal ca	Ductal ca
37.	Lakshmi	11789	67	Ca breast	Ductal ca	Ductal ca	Ductal ca
38.	Murugeswari	18854	20	Fibroadenoma	Fibroadenoma	Fibroadenoma	Fibroadenoma
39.	Subbathal	17828	70	Ca breast	Ductal ca	Ductal ca	Ductal ca
40.	Meenatchi	18278	40	Phyllodes tumour	Giant fibroadenoma	Fibroadenoma	Benign Phyllodes tumour
41.	Sandhubee	15542	50	Ca breast	Ductal ca	Ductal ca	Invasive ductal ca
42.	Maheswari	21439	25	Fibroadenoma	Fibroadenoma	Fibroadenoma	Fibroadenoma
43.	Jothimani	21260	23	Fibroadenoma	Fibroadenoma	Fibroadenoma	Fibroadenoma
44.	Rani	19617	17	Fibroadenoma	Fibroadenoma	Fibroadenoma	Fibroadenoma
45	Indirani	12873	53	Ca breast	Ductal ca	Ductal ca	Invasive ductal ca
46.	Maragatham	29542	44	Fibroadenoma	Fibroadenoma	Fibroadenoma with epitheliosis	Fibroadenoma
47.	Kuttiyammal	26641	50	Ca breast	Colloid ca	Ductal ca	Colloid ca
48.	Ponnuthai	28049	45	Phyllodes tumour	Malignant phyllodes	Malignant phyllodes	Metaplastic ca
49.	Devamani	27986	36	Ca breast	Fibroadenoma	Fibroadenoma	Fibroadenoma
50.	Jothi	27985	60	Ca breast	Ductal ca	Ductal ca	Ductal ca